

AMENDMENTS TO THE CLAIMS:

Claims 1-29 are canceled without prejudice or disclaimer. Claims 30-49 are added. The following is the status of the above-captioned application as amended.

Claims 1-29 (Canceled)

Claim 30 (New). A bacterial host cell comprising at least two copies of an amplification unit in its genome, said amplification unit comprising:

- i) at least one copy of a gene of interest, and
- ii) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity substantially lower than the endogenous promoter of said conditionally essential gene, and wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source.

Claim 31 (New). The cell of claim 30, wherein the bacterial cell is a prokaryotic cell.

Claim 32 (New). The cell of claim 31, wherein the bacterial prokaryotic cell is a Gram-positive cell.

Claim 33 (New). The cell of claim 32, wherein the bacterial Gram positive cell is a species of the genus *Bacillus*.

Claim 34 (New). The cell of claim 30, wherein the gene of interest encodes an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase.

Claim 35 (New). The cell of claim 30, wherein the gene of interest encodes an antimicrobial peptide.

Claim 36 (New). The cell of claim 30, wherein the gene of interest encodes a peptide with biological activity in the human body.

Claim 37 (New). The cell of claim 30, wherein the conditionally essential gene encodes an enzyme from the biosynthetic pathway of an amino acid.

Claim 38 (New). The cell of claim 37, wherein the conditionally essential gene encodes one or more polypeptide(s) involved in lysine, leucine or methionine synthesis.

Claim 39 (New). The cell of claim 37, wherein the conditionally essential gene is at least 75% identical to the *lysA* sequence of *Bacillus licheniformis* shown in SEQ ID NO:48 of WO 02/00907 A1, the *leuB* sequence of *Bacillus licheniformis*, the *metC* sequence of *Bacillus licheniformis* shown in SEQ ID NO:42 of WO 02/00907 A1, or the *metE* sequence of *Bacillus subtilis* shown in positions 997 to 2199 of SEQ ID NO:16.

Claim 40 (New). The cell of claim 30, wherein the conditionally essential gene encodes a glutamyl-tRNA reductase.

Claim 41 (New). The cell of claim 30, wherein the conditionally essential gene is at least 75% identical to the *hemA* sequence of *Bacillus licheniformis*.

Claim 42 (New). The cell of claim 30, wherein the conditionally essential gene encodes an enzyme required for xylose utilization.

Claim 43 (New). The cell of claim 30, wherein the conditionally essential gene encodes a xylose isomerase and is at least 75% identical to the *xylA* gene of *Bacillus licheniformis*.

Claim 44 (New). The cell of claim 30, wherein the conditionally essential gene encodes an enzyme required for gluconate utilization.

Claim 45 (New). The cell of claim 30, wherein the conditionally essential gene encodes a gluconate kinase (EC 2.7.1.12) or a gluconate permease or both and is at least 75% identical to any of the *gntK* and *gntP* sequences of *Bacillus licheniformis*.

Claim 46 (New). The cell of claim 30, wherein the conditionally essential gene encodes an enzyme required for glycerol utilization.

Claim 47 (New). The cell of claim 30, wherein the conditionally essential gene encodes a glycerol uptake facilitator (permease), a glycerol kinase, or a glycerol dehydrogenase, and is at least 75% identical to any of the *glpP*, *glpF*, *glpK*, and *glpD* sequences of *Bacillus licheniformis*.

Claim 48 (New). A method for producing a protein encoded by a gene of interest, comprising
a) culturing a bacterial host cell comprising at least two duplicated copies of an amplification unit in its genome, the amplification unit comprising:

- i) at least one copy of the gene of interest, and
- ii) an expressible conditionally essential gene, wherein the conditionally essential gene is either promoterless or transcribed from a heterologous promoter having an activity substantially lower than the endogenous promoter of said conditionally essential gene,

wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source; and

- b) recovering the protein.

Claim 49 (New). A method for producing a bacterial cell comprising two or more amplified chromosomal copies of a gene of interest, the method comprising:

- a) providing a bacterial cell comprising at least one copy of an amplification unit, the unit comprising:

- i) at least one copy of the gene of interest, and
- ii) an expressible functional copy of a conditionally essential gene, which is either promoterless or transcribed from a heterologous promoter having an activity substantially lower than the endogenous promoter of said conditionally essential gene,

wherein the conditionally essential gene if not functional would render the cell auxotrophic for at least one specific substance or unable to utilize one or more specific sole carbon source;

- b) cultivating the cell under conditions suitable for growth in a medium deficient of said at least one specific substance and/or with said one or more specific sole carbon source, thereby providing a growth advantage to a cell in which the amplification unit has been duplicated in the chromosome; and

c) selecting a cell wherein the amplification unit has been duplicated in the chromosome, whereby two or more amplified chromosomal copies of the gene of interest were produced.